Energy acquisition and allocation in an ectothermic predator exposed to a common environmental stressor

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Abstract

Stressors are commonly encountered by organisms and often prove to be energetically costly. Certain stressors can simultaneously affect multiple components of an animal's energy budget and can either exacerbate energetic costs to the individual or offset one another. Here we used a commonly encountered stressor, the pesticide carbaryl, to examine the complex effects that acute environmental disturbances can have on energy expenditure, allocation, and acquisition, important processes that influence growth and reproduction. After exposing lizards (Sceloporus occidentalis) to carbaryl, we measured their metabolism over a 48 h period and assessed their food consumption over 96 h. We found no difference in total energy expenditure among treatment groups, but lizards exposed to the highest dose of carbaryl allocated energy differently than other groups. Compared to controls, these lizards exhibited a 16–30% increase in standard metabolic rate (SMR), which was offset by a 45–58% decrease in additional energy expenditures. Lizards in the highest dose group also exhibited a 30–34% decrease in energy acquisition compared to controls. The net result was a 1.83 kJ decrease in energy assimilation, equivalent to 5 times their daily SMR requirements. Our results indicate that energetic consequences of stressors may result in complex energetic trade-offs, and emphasize the need to simultaneously examine the effect of stressors on multiple portions of an animal's energy budget.
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1. Introduction

Throughout the life of an animal, assimilated energy is allocated towards two competing demands, maintenance and production (Congdon et al., 1982, 2001; McNab, 2002). Maintenance costs are essential for the continuity of life and include both standard maintenance (i.e., basal energy costs for living) and activity maintenance (e.g. energy necessary for foraging, circadian rhythms, and digestion), whereas energy allocated towards production supports important life history processes such as growth and reproduction (Congdon et al., 1982).

For many organisms a large proportion of assimilated energy is allocated towards maintenance costs, but substantially less is allocated towards production. For example, in reptiles, approximately 80% of their energy budget is comprised of maintenance costs, whereas production accounts for the remaining 20% (Congdon et al., 1982). Even small increases in maintenance can result in proportionately large decreases in the energy available for growth and reproduction. During times of limited energy availability, energetic costs necessary for living are met, while energy allocated towards high levels of activity and production are often restricted (Lucas, 1996; McNab, 2002).

Stress, a phenomenon all animals regularly experience, is one factor that can be energetically costly for organisms. McEwen and Wingfield (2003) suggested that stressors increase the energetic burden to the animal, and when this burden exceeds the energy an animal can acquire from the environment,

the deleterious effects of stress become apparent. Stressors can be energetically costly for a variety of reasons, including the deployment of processes that protect against stressors (e.g., synthesis of heat-shock proteins; Krebs and Loeschcke, 1994), or rid the body of a stressor (e.g., metabolizing and excreting contaminants; Calow, 1991), and changes in stress hormone concentrations (Morgan and Iwama, 1996). Stressors may also decrease energy assimilation efficiency (Baird et al., 1990), alter the accessibility of energy in the environment (Hopkins et al., 2004a), and influence energy expenditures associated with activity (Congdon et al., 2001).

An extensive number of studies have addressed the energetic cost of both natural (e.g., Barton and Schreck, 1987; Christiansen et al., 1991; duPreez et al., 1996; Davis and Schreck, 1997; Iwama et al., 1997; Dalla Via et al., 1998; Harris et al., 1998; Sloman et al., 2000; Ricklefs and Williams, 2003) and anthropogenic (Rowe et al., 1998, 2001; Hopkins et al., 1999, 2002; Bain et al., 2004) stressors, however, the impact of the stressor on energy expenditure varies greatly among species and types of stressors. Calow (1991) suggested that the difference in the energetic costs of stressors may result from the effects these stimuli have on different biological processes. For example, some processes may be downregulated in response to a stressor, while others might be upregulated, serving to offset energetic costs in some instances and exacerbating costs in others. However, this conceptual framework has rarely been tested directly. Hopkins et al. (2002) noted that benthic fish with restricted access to food resources exhibited the typical hypometabolic response observed in animals facing food deprivation (Wang et al., 2006). However, when food-deprived fish were exposed to an additional stressor (metal-contaminated sediments), the fish failed to reduce their metabolism, which resulted in reduced body condition and survivorship compared to fish in reference conditions (Hopkins et al., 2002). The author's suggested that tissue repair and/or upregulation of physiological processes such as metabolism and excretion of contaminants could account for the apparent inability of contaminant-exposed individuals to restrict their energy usage during times of reduced resource abundance. In another example, Barber et al. (1990) expected to find an increase in O_2 consumption in Daphnia magna after exposure to cadmium and dicholoranaline because of evidence for deployment of energetically costly stress-resisting mechanisms after exposure to these contaminants (Baird et al., 1990). Although there was no significant change in total O_2 consumption, the authors noted a reduction in food intake and inferred that a rise in basal metabolism most likely did occur but was offset by a decrease in metabolic costs associated with consuming and digesting food (Barber et al., 1990). These two previous studies suggest that patterns of energy expenditure, allocation, and acquisition under stressful conditions can be complex and that simultaneous monitoring of several parameters related to energy use can provide insight into the ultimate energetic consequences of a stressor to an individual.

To further probe the complex effects of stressors on animals' energy budgets, we chose to study the chemical carbaryl, one of the most widely used non-commercial insecticides which also has a well-defined mode of toxicity. Carbaryl inhibits the breakdown of acetylcholine (ACh), a neurotransmitter found at neuromuscular junctions, by deactivating the enzyme acetyl-cholinesterase (AChE) (Fukuto, 1990). Carbaryl is among the least toxic pesticides in its class and thus is used for a wide variety of applications (Hill, 2003). According to the US EPA's Pesticide Industry Sales and Usage 2000–2001 Market Report (Kiely et al., 2004), carbaryl was ranked as the sixth most commonly used conventional pesticide in the home and garden market. Because carbaryl and other AChE-inhibiting pesticides are very short-lived in the environment (Half-life in soil 8–18 days; Nkedi-Kizza and Brown, 1998), pulse exposures representing acutely stressful events are expected to be more ecologically relevant encounters than low-level chronic exposure typical of some other environmental pollutants (e.g., metals).

In the present study we determined the effects of this commonly encountered environmental stressor on energy acquisition, expenditure, and allocation in a lizard. We quantified total energy consumption and how much of this energy was consumed to support basal metabolism for 48 h after exposure to carbaryl. We also examined whether exposure to the stressor would alter energy acquisition because a compensatory increase in food consumption could theoretically alleviate energetic costs of the stressor. Alternatively, a decrease in energy acquisition could exacerbate any energetic costs of the stressor and further compromise an individual's energy balance. Measuring multiple metabolic parameters and energy acquisition allowed us to explore the energetic tradeoffs that potentially occur in animals experiencing stress.

2. Materials and methods

2.1. Fence lizard natural history and husbandry

Sceloporus lizards belong to the family Phrynosomatidae which accounts for more than 30% of all lizard species in the United States. Some Sceloporus serve as good study organisms because their entire lifecycle is manageable in the laboratory, and a great deal is known about their ecology, physiology, performance and life history (e.g., Garland et al., 1990; Sinervo, 1990; Angilletta et al., 2002; Talent et al., 2002; Roe et al., 2005). The species used in this study, the western fence lizard (Sceloporus occidentalis), ranges from Mexico to Canada between the California coast and western Utah, USA. The original parental stock of our laboratory subjects originated from a population in the grasslands of the San Joaquin Valley, CA, USA. Most females from this population reach sexual maturity within one year under ad libitum feeding conditions in the laboratory, and lay 3–6 clutches of 8–15 eggs per year. This population of western fence lizards does especially well under laboratory conditions and has been identified as a good candidate for use as a laboratory reptile model (Talent et al., 2002; Hopkins et al., 2005b).

Juvenile western fence lizards were shipped to The Savannah River Ecology Laboratory (SREL) from a breeding colony at Oklahoma State University. Lizard husbandry was identical to

Hopkins et al. (2005a,b) with the following exceptions: a 10:14 (light:dark) photoperiod, a daytime temperature gradient of ~28–40 °C, and a diet consisting of 4 crickets (~1.5 cm each) per day. A total of 117 lizards were used in the following experiments and no lizard exposed to carbaryl was used in additional experiments. However, some control lizards were used in both experiments to reduce the number of lizards sacrificed during our studies. Control lizards used in both experiments were given at least two weeks to recover from the first experiment and then were evenly distributed among the three pesticide treatment groups in the second experiment. Experimental procedures were approved by the University of Georgia IACUC (A2004-10049-0).

2.2. Experiment I: Energy expenditure and allocation

In the first experiment we determined the effect of carbaryl on lizard metabolism. We simultaneously measured both the volume of oxygen consumed (V_{O_2}) and volume of carbon dioxide produced (V_{CO_2}) every 1.18 h over a 48 h period for a total of 38–39 measurements using a computer-controlled closed circuit respirometer (Micro-Oxymax, Columbus Instruments Columbus, OH, USA) following methods similar to Hopkins et al. (2004a,b) and Roe et al. (2005). Lizards were post-absorptive (i.e., fasted 48 h) during all respirometry trials. After fasting lizards were assigned to one of five treatment groups; control, gavage control, 2.5 µg/g, 25 µg/g, or 250 µg/g carbaryl. Lizards were then weighed and administered their respective treatment. After treatment administration lizards were placed in individual respirometry chambers (1000 mL Erlenmeyer flask) covered with paper to visually isolate them from external stimuli; the respirometry chambers were then placed in an environmental chamber at 30 °C for the duration of the trial. All trials were conducted between March and August of 2004. Each treatment group was comprised of six lizards per sex. However, a male in the highest treatment group died 41 h after being exposed to carbaryl. To equalize sample sizes among treatments, we replaced this individual with another male.

Carbaryl was administered to lizards via oral gavage using an Eppendorf micro-pipette (2–20 µL). The 2.5 and 25 µg/g solutions were made within 1 h of administration by diluting a 22.5% formulation of carbaryl (Sevin®; Garden Tech, Lexington, KY, USA) with water and then vortexing the solution for 1 min. Lizards in the 250 µg/g treatment group received an undiluted dose of the 22.5% Sevin® formulation and lizards in the oral gavage treatment group received a comparable volume of water. Control lizards were not manipulated. All lizards used in the respirometry trials weighed between 6.3 and 9.1 g and dose volumes were extremely low, ranging between 6.5 and 9.4 µL.

Although contaminated insects are an important route of exposure to pesticides for insectivorous vertebrates inhabiting areas that receive pesticide application, very little data exists on invertebrate pesticide residues. Therefore, it is difficult to predict actual concentrations lizards are likely to encounter in the wild. In the only study to directly quantify carbaryl residues of terrestrial invertebrates, Fair et al. (1995), showed that

grasshoppers had mean residues of 17 µg/g 2 days following rangeland application of 0.5 kg active ingredient/ha. Using this insect residue data and carbaryl application rates, which can vary from 1.12 to 22.42 kg active ingredient/ha (EPA, 2004) we estimated that a 10 g lizard consuming 1 g of prey could ingest dose concentrations ranging between 3.9 and 78.5 µg/g 2 days following carbaryl application. We selected three doses that fully encompass the range of concentrations that lizards could encounter in the environment.

Using the respirometry data collected we quantified three metabolic parameters: 1) total V_{O_2} and V_{CO_2}, 2) V_{O_2} and V_{CO_2} to support standard metabolic rate (SMR), and 3) the amount of gas exchanged to support metabolic activity exceeding SMR (e.g., spontaneous activity, circadian rhythms; hereafter, referred to as additional V_{O_2} and V_{CO_2}). Total V_{O_2} and V_{CO_2} were estimated as the integral (i.e., area under the curve) of respiration (V_{O_2} and V_{CO_2}) over the entire respirometry trial. Because SMR is the metabolic rate of a resting, post-absorptive ectotherm at a specified temperature during the inactive phase of its circadian cycle (Bennett and Dawson, 1976), we estimated SMR as the lowest quartile value from each individual's respiratory profile, a
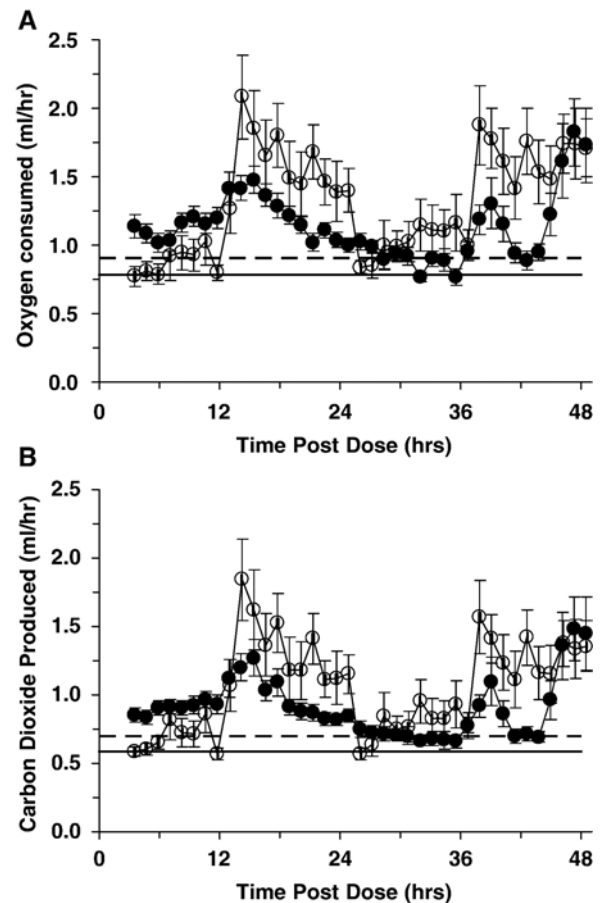

Fig. 1. Mean (±1SE) oxygen consumption (A) rates (mL/h) and carbon dioxide production (B) rates in western fence lizards (*Sceloporus occidentalis*, 6.3–9.1 g) at 30 °C acutely exposed to 250 µg/g carbaryl (closed circles) compared to controls (open circles) over a 48 h period. The dashed line and solid line represent SMR for lizards exposed to 250 µg/g carbaryl and control lizards, respectively. $N=6$ lizards/sex/treatment.

procedure that removes peaks in respiration associated with activity and circadian rhythms. Other studies on reptiles have been successful at estimating SMR using similar methods (Litzgus and Hopkins, 2003; Hopkins et al., 2004a,b; Roe et al., 2005; Hare et al., 2006). V_{O_2} and V_{CO_2} to support SMR were estimated as the integral of SMR. Additional V_{O_2} and V_{CO_2} were estimated by subtracting the integral of SMR from the total V_{O_2} and V_{CO_2}. Total respiration, additional respiration, and respiration to support SMR were then converted to units of energy expended (1 mL O_2 consumed $= 19.8$ J; Secor and Diamond, 1995). We also determined respiratory quotients (RQ: the ratio of CO_2 produced to O_2 consumed) for each lizard to allow inference about the substrates used for aerobic catabolism (Withers, 1992).

2.3. Experiment II: Energy acquisition

In the second experiment, we sought to determine whether exposure to carbaryl affected food consumption. After fasting lizards for 48 h, we then assigned them to one of the five aforementioned treatment groups, their masses were recorded, and their respective treatment was administered via oral gavage.
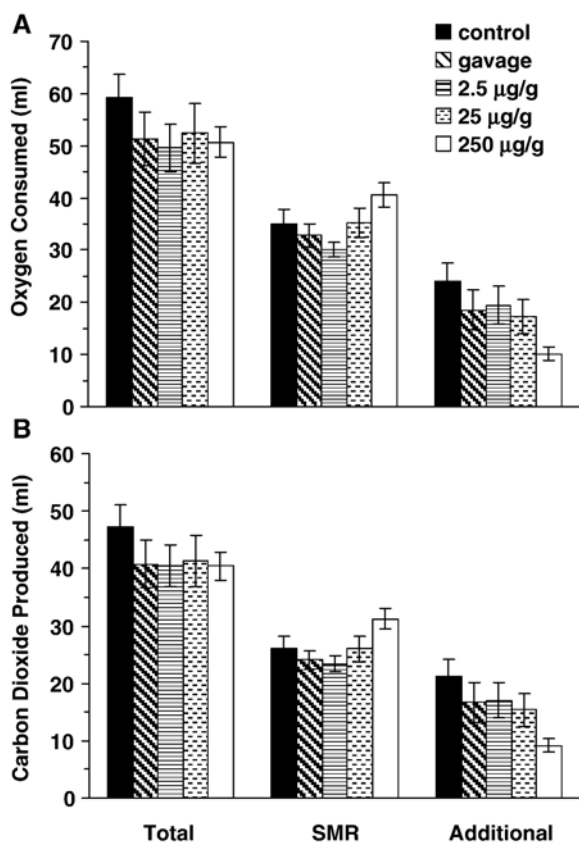

Fig. 2. Patterns of oxygen consumption (A) (mL) and carbon dioxide production (B) among western fence lizards (*Sceloporus occidentalis*) acutely exposed to varying concentrations of carbaryl. Total oxygen consumed over a 48 h period (Total) following carbaryl administration is partitioned into oxygen consumed to support standard metabolic rate (SMR) and that consumed to support additional energy expenses (Additional). Data are presented as means±1SE. $N=6$ lizards/sex/treatment.
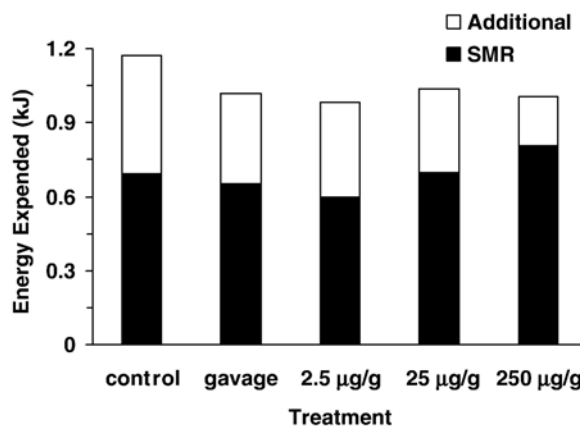

Fig. 3. Summary of energy consumption (kJ) patterns among western fence lizards (*Sceloporus occidentalis*) acutely exposed to varying concentrations of carbaryl. Total energy expended is allocated to either standard metabolic rate (solid portion of bar) or to support additional energy expenses (open portion). $N=6$ lizards/sex/treatment.

During feeding trials, lizards were housed individually in a $52 \times 36 \times 18$ cm plastic cage with a screen lid arranged in a rack system. At 24 and 96 h post dose administration (repeated measures on individuals), each lizard was offered 10 crickets weighing (in total) 15% of the lizard's body mass. Twenty-four hours following each feeding trial, all remaining crickets were removed, counted, and weighed. Lizards were not fed during the 48 h between the 24 and 96 h feeding trials. Lizard masses in this experiment ranged between 6.1–9.5 g and dose volumes ranged between 6.2–9.8 μL. Treatment groups were comprised of eight lizards per sex. Feeding trials were conducted between March–May of 2004.

To determine the energy content of prey items, three composite samples of crickets were lyophilized and ground into a powder. Energy content of homogenized samples was determined using an adiabatic bomb calorimeter (Parr instrument Co., Moline IL, USA) at the Poultry Science Research Laboratory of the University of Georgia.

2.4. Statistical analyses

We examined the influence of treatment on total respiration, additional respiration, and respiration to support SMR (V_{O_2} and V_{CO_2} for each parameter) using a multivariate analysis of variance (MANOVA). Wilks' lambda statistical values were used to assess statistical significance. All respiratory variables and mass were $\log_{10}$-transformed to better approximate assumptions of the model. In the model we considered treatment and sex as independent variables and $\log_{10}$-transformed mass as the covariate. The initial model included all interaction terms but insignificant interactions were dropped in subsequent iterations of the model. Initial models indicated there was no difference in respiration between males and females, and therefore sexes were combined for statistical analyses. Individual ANCOVAs for each dependent variable were also examined to determine which variables contributed to significant effects in the multivariate model.
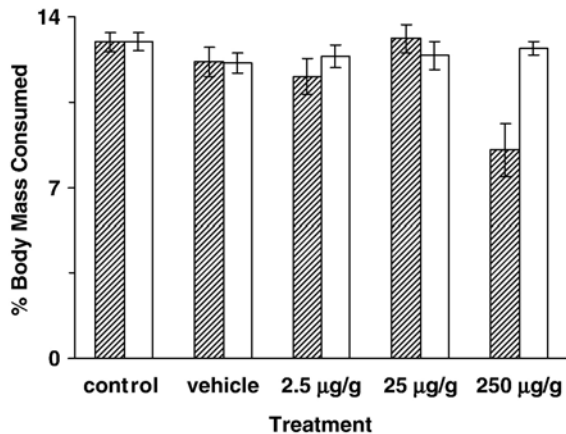
Fig. 4. Patterns of food consumption, calculated as the percentage of the individual's body mass it consumed in crickets, in western fence lizards (*Sceloporus occidentalis*) after acute exposure to varying concentrations of carbaryl. Lizards were offered 15% of their body mass during each trial. Hatched and open bars represent 24 and 96 h feed trials, respectively. Data are presented as means ± 1SE. $N = 8$ lizards/sex/treatment.

Because the variance associated with RQ values was not equivalent among treatments, we compared RQs among treatments using a mixed model approach to ANCOVA (SAS PROC MIXED). Log$_{10}$-transformed mass was treated as a covariate and RQ values were arc-sin square root transformed to better approximate a normal distribution. Because RQ was not independent of the other respiratory variables, we applied a sequential bonferroni adjustment to account for multiple, non-independent comparisons.

To examine the influence of treatment on food consumption we conducted a repeated measures ANOVA using a mixed model approach (SAS PROC MIXED). The percentage (arc-sin transformed) of a lizard's body mass consumed in crickets was treated as the dependent variable, treatment and sex were treated as independent variables, and time was the repeated variable. Initial models indicated there was no difference in food consumption between males and females ($p = 0.39$), therefore sexes were combined for statistical analyses. Because we were interested in whether lizards would recover from exposure over time, we also included the interaction between treatment and time in the model.

3. Results

Oxygen consumption and carbon dioxide production of lizards were influenced by treatment (Wilks' lambda = 0.46; $F_{24,172} = 1.8$; $p = 0.019$; Fig. 1), but examination of individual ANCOVA's for each respiratory parameter revealed that treatment effects were dependent upon which parameter was considered (Figs. 2 and 3). Treatment had no effect on total V_{O_2} or V_{CO_2} ($p > 0.582$). However, V_{O_2} and V_{CO_2} to support SMR were significantly affected by treatment, namely a 16–30% increase in gas exchange to support SMR (equivalent to 0.11–0.14 kJ) in the highest treatment group compared to the two control groups (V_{O_2}: $F = 2.9$, $p = 0.029$; V_{CO_2}: $F = 2.9$, $p = 0.030$). Furthermore, treatment had a significant effect on

additional V_{O_2} ($F = 2.6$, $p = 0.047$) and a marginal effect on additional V_{CO_2} ($p = 0.061$). This resulted from a 45–58% decrease in additional V_{O_2} and V_{CO_2} in the highest treatment group compared to controls (equivalent to 0.15–0.28 kJ). However, treatment had no effect on RQ (range of mean RQs = 0.77–0.81; $p = 0.615$); suggesting that lizards in all treatment groups were metabolizing similar substrates to meet their energy requirements.

There was a significant effect of treatment on food consumption, but this effect was dependent upon time (treatment: $F_{4,75} = 2.94$, $p = 0.03$; time: $F_{1,75} = 6.53$, $p = 0.01$; treatment × time: $F_{4,75} = 7.01$, $p < 0.001$). The highest treatment group exhibited a 30–34% decrease in food consumption compared to controls, but recuperated within 96 h (Fig. 4). Energy content of crickets was $5.8 ± 0.01$ kJ/g of cricket.

4. Discussion

Our study is among the first to demonstrate that stress-induced changes in energy allocation to multiple portions of the energy budget can offset one another. We found that while total energy expended did not differ among treatments, lizards exposed to the highest dose of carbaryl allocated energy differently than lizards in other groups (Fig. 3). Specifically we found that lizards in the highest dose group allocated 16–30% more energy towards SMR than control groups (Fig. 2), but 45–58% less energy towards additional energy expenditures (Fig. 2). Alterations in energy allocation after acute exposure to carbaryl were sustained for at least 44 h (Fig. 1). In addition to altered energy allocation, lizards in the highest dose group also exhibited a 30–34% decrease in food consumption during the 24 h feeding trial compared to controls. Thus, although changes in energy allocation were compensatory, lizards still experienced energetic consequences from exposure to the stressor due to decreased energy acquisition.

An increase in SMR and an apparent decrease in spontaneous activity appear to be the two primary effects that offset one another. Processes such as the release of stress hormones (Wendelaar Bonga, 1997), breakdown and excretion of contaminants (Calow, 1991), the effects of carbaryl on muscle fasciculation, and perhaps the direct effects of AChE-inhibitors on the respiratory system (Grue et al., 1991) can be energetically costly and may have contributed to the increase in SMR observed in lizards in the highest dose group. The temporal patterns in gas exchange (Fig. 1) suggest that decreased spontaneous activity resulted in the decrease in additional energy expenditure and is consistent with the results of two other studies that noted decreased activity in leopard frog tadpoles (Bridges, 1997) and red-winged blackbirds (Brunet et al., 1997) after exposure to AChE-inhibitors. Taken together, our findings support the idea that environmental stressors simultaneously can have complex and differing effects on the various components of metabolism (Calow, 1991).

A substantial amount of research exists on the effects of stress on appetite and food intake. The bulk of which indicates that severe stress reduces food ingestion (Carr, 2002), thereby negatively impacting an individual's energy budget through decreased energy acquisition. In our study, we found that

although energy allocations were offsetting, lizards exposed to the highest dose of carbaryl would still experience negative energetic consequences compared to controls due to a 30–34% reduction in food consumption (Fig. 4). For a 10 g lizard, this period of prandial quiescence would result in 440 mg less food being consumed compared to a similarly sized control lizard. Assuming that 72% of the energy in the ingested food would be assimilated (Angilletta, 2001), we estimate that lizards exposed to high concentrations of carbaryl could experience a 1.837 kJ/day decrease in energy assimilation compared to controls. This net decrease would be significant, exceeding our estimates of the daily standard maintenance requirements of lizards (0.35 kJ/day) by 5-fold. It is worth noting that energy allocation and clinical symptoms (e.g., muscle twitching) were altered for almost 48 h. Because we did not continuously monitor food consumption for the entire 48 h period, it is possible that our calculations are an underestimate of the effect of carbaryl on energy acquisition (Figs. 1 and 4).

Although changes in energy allocation and acquisition were short-lived, which is consistent with the biochemical properties of carbaryl (Fukuto, 1990) and the duration of its biological effects (Hopkins et al., 2005a; Hopkins and Winne, 2006; DuRant et al., in press), wildlife are exposed to this and many other stressors in repeated pulses over each active season (Davidson et al., 2001). In addition, stressors that are persistent in the environment, such as social stress, disease, and invasive species, could result in extended periods of altered energy expenditure and/or energetic trade-offs. In fact, such extended alterations in metabolism have been noted in animals exposed to heavy metals, a persistent environmental stressor (Hopkins et al., 1999; Rowe et al., 2001). As suggested by McEwen and Wingfield (2003) animals experiencing negative energy balance may enter an emergency life history stage to regain favorable energy relations. The emergency life history stage is a temporary interruption of an animal's normal life cycle in which the animal's behavior and physiology are directed towards survival and away from energetically expensive processes like reproduction (Wingfield et al., 1998). Serious energetic trade-offs and periods of elevated energy expenditure could result in the triggering of an emergency life history stage, and may cause the individual to forego important life history events, such as reproduction.

Even though we observed no net change in total energy expenditure, our results indicate that changes in energy allocation associated with stress can be dynamic and complex. All else being equal, an increase in energy use for one portion of the energy budget will cause an energy shortfall in others. In most previous studies, an increase in basic maintenance requirements caused by stress (increase in SMR or BMR) has been suggested to detract from the energy available for production (e.g., Hopkins et al., 1999). Here we demonstrate that moderation in activity may be sufficient to offset these costs. However, decreased spontaneous activity also has consequences for lizards because it could alter susceptibility to predation (Weis et al., 2001), decrease foraging, and possibly decrease digestive performance given the importance of basking to lizard digestion (Angilletta, 2001). In the end, sustained changes in energy allocation and acquisition could ultimately result in decreased growth and reproduction. However,

additional studies on repeated acute stress or sustained stress are needed to understand the implications of these effects on the production portion of an individual's energy budget.

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